Profiling of Oncogenic Driver Events in Lung Adenocarcinoma Revealed MET Mutation as Independent Prognostic Factor

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Introduction: Oncogenic driver mutations activating receptor tyrosine kinase pathways are promising predictive markers for targeted treatment. We investigated the mutation profile of an updated driver events list on receptor tyrosine kinase/RAS/PI3K axis and the clinicopathologic implications in a cohort of never-smoker predominated Chinese lung adenocarcinoma.

Methods: We tested 154 lung adenocarcinomas and adenosquamous carcinomas for EGFR, KRAS, HER2, BRAF, PIK3CA, MET, NRAS, MAP2K1, and RIT1 mutations by polymerase chain reaction-direct sequencing. MET amplification and ALK and ROS1 translocations were assessed by fluorescent in situ hybridizations. MET and thyroid transcription factor-1 protein expressions were investigated by immunohistochemistry.

Results: Seventy percent of lung adenocarcinomas carried actionable driver events. Alterations on EGFR (43%), KRAS (11.4%), ALK (6%), and MET (5.4%) were frequently found. ROS1 translocation and mutations involving BRAF, HER2, NRAS, and PIK3CA were also detected. No mutation was observed in RIT1 and MAP2K1. Patients with EGFR mutations had a favorable prognosis, whereas those with MET mutations had poorer overall survival. Multivariate analysis further demonstrated that MET mutation was an independent prognostic factor. Although MET protein expression was detected in 65% of lung adenocarcinoma, only 10% of the MET-immunohistochemistry positive tumors harbor MET DNA alterations that drove protein overexpression. Appropriate predictive biomarker is essential for selecting patients who might benefit from specific targeted therapy.

Conclusion: Actionable driver events can be detected in two thirds of lung adenocarcinoma. MET DNA alterations define a subset of patients with aggressive diseases that might potentially benefit from anti-MET targeted therapy. High negative predictive values of thyroid transcription factor-1 and MET expression suggest potential roles as surrogate markers for EGFR and/or MET mutations.

Key Words: Lung cancer, EGFR, KRAS, ALK, HER2, BRAF, MET, ROS1, RIT1, TTF-1 lung adenocarcinoma, NRAS, Driver mutations, Oncogenic driver mutation, East Asian lung cancer, MET amplification, Targeted therapy, Genetic marker, Biomarker, Molecular classification lung cancer.

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Lung cancer is the leading cause of cancer-related death worldwide.[1] Over 98% of the lung cancers are carcinomas, including small-cell carcinoma and non–small-cell carcinoma (NSCLC). NSCLC is further classified into adenocarcinoma, squamous cell carcinoma, and large-cell carcinoma according to the pathological characteristics. Driver mutations activating the receptor tyrosine kinase (RTK) pathway have been found in more than 70% of the lung adenocarcinomas that can predict benefit from targeted therapies. Clinically, targeted agents against EGFR, ALK,[2–6] RET,[7] and ROS1[8,9] have dramatically improved the treatment outcome in patients with specific driver mutations. Other targeting therapies against BRAF,[10–12] ERBB2,[13–16] and PIK3CA are under investigations. Efforts in next generation sequencing studies have revealed rarer driver mutations including NRG1,[17,18] NTRK,[19] ERBB4,[18] BRAF[18] fusion and MET,[20–22] ARAF,[23] CRAF,[23] RIT1,[22,24] HRAS,[25,26] and NRAS[27,28] mutations that may serve as potential therapeutic targets. The number of predictive biomarkers with their matched targeted drugs entering clinical trials is expected to increase dramatically. This study aimed to comprehensively investigate the mutational profile of an updated list of diver genes on RTK/RAS/PI3K axis in a cohort of lung adenocarcinoma from Chinese ethnicity.

MATERIALS AND METHODS

Clinical Samples

The study cohort was a retrospective cohort retrieved from the pathology archive. It recruited patients with (1) lung

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cancer undergoing surgical resection from December 1998 to December 2010 at Prince of Wales Hospital, Hong Kong, (2) histological diagnosis of adenocarcinoma or adenosquamous carcinoma, and (3) availability of frozen tissue. Specimens were collected with informed consent from the patients. The study protocol was approved by the Joint CUHK-NTE Clinical Research Ethics Committee, Hong Kong. All specimens were reviewed by a pathologist (K.F.T.) to confirm the histological diagnosis and tumor cell content. Patients' demographic data and clinicopathological characteristics were obtained from medical records. The disease stage was determined according to the 7th edition of American Joint Committee on Cancer TNM classification system. The smoking status was either categorized into never-smoker (individuals smoke less than 100 cigarettes in their life-time) or ever-smoker (individuals smoke more than 100 cigarettes in their life-time).[22] Patients with history of chemotherapy before surgery, demographic data missing, or nonprimary tumor were excluded. After surgical resection, all patients were followed up according to institutional practice. The duration of follow-up was calculated from the date of surgical operation to the date of the last follow-up (cut-off at June 30, 2014) or death. Disease-specific overall survival was defined from the time of surgery to the time of cancer-related death. Relapse-free survival was defined from the time of curative surgery to the time of radiological evidence of tumor relapse.

Microdissection and DNA Extraction

DNA was extracted from both frozen and formalin-fixed paraffin-embedded tumor tissues using QIAamp DNA mini kit (QIANGEN, Valencia, CA) according to the manufacturer's instruction. Manual microdissection was performed to ensure more than 50% tumor content in each DNA sample for subsequent analysis.

Mutational Analysis

EGFR exon 18–21, KRAS exon 2 and 3, HER2 exon 20, BRAF codon 600 and 601, MET exon 14 and intron boundaries, MAP2K1 exon 2, PIK3CA exon 9 and 20, NRAS exon 2 and RIT1 exon 4 and 5 were screened by polymerase chain reaction (PCR) and direct sequencing. PCR primer sequences, annealing temperatures, and cycling conditions were listed in Supplementary Table 1 (Supplemental Digital Content 1, http://links.lww.com/JTO/A853). The PCR products were purified and sequenced using the BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster city, CA) and analyzed by the Life Technologies 3130xl Genetic Analyzer. The data were collected and analyzed using Applied Biosystems sequencing analysis software. The DNA sequence was compared with consensus coding sequences from the Consensus Coding DNA Sequnce Database.

Construction of Tissue Microarray

Tissue microarrays were constructed using a tissue arrayer (Beecher Instruments, Silver Spring, MD). The location of tumor area on the donor formalin-fixed paraffin-embedded tissue block was first marked on the hematoxylin and eosin-stained histological section. Three representative 1-mm cores were obtained from each tumor and inserted to a recipient paraffin block. For fluorescent in situ hybridization (FISH) and immunohistochemistry (IHC), 4-μm tissue sections were prepared and mounted onto Superfrost Plus microscope slides.

Fluorescent In Situ Hybridization

ALK and ROS1 translocation status were investigated by using commercial break apart FISH probes: Vysis LSI ALK Dual Color Break Apart Rearrangement Probe (Abbott Molecular, Abbott Park, IL) and Aquarius ROS1 Break Apart probe (Cytocell, Cambridge, GB). At least 100 informative tumor nuclei were examined. Only nuclei showing both green and red signals were considered informative. Positive ALK or ROS1 rearrangements were defined as more than 15% of the tumor cells carried break apart signals. MET gene copy number/amplification status was investigated by MET/CEP7 FISH probe (Abbott Molecular, IL). Copy number per cell and MET/CEP7 ratio were counted in at least 50 nonoverlapping tumor cell nuclei. MET amplification was defined by MET/CEP7 ratio greater than or equal to 2.

Immunohistochemistry

IHC was carried out using Benchmark XT autostainer (Ventana, Tucson, AZ) using Ultraview detection system. MET IHC was performed using Confirm anti-total c-MET (SP44) rabbit monoclonal antibody (Ventana, Tucson, AZ) according to manufacturer's instruction. Expression level of MET protein was determined by a scoring system considering both staining intensity and prevalence of intensities in tumor cells. The four staining score were defined as following: 3+ (≥50% of tumor cells staining with strong intensity); 2+ (≥50% of tumor cells with moderate or higher staining but <50% with strong intensity); 1+ (≥50% of tumor cells with weak or higher staining but <50% with moderate or higher intensity); or 0 (no staining or <50% of tumor cells with any intensity).[29] Tumors with moderate to strong MET protein expression (scores 2+ and 3+) were considered positive, whereas scores 0 and 1+ were regarded as negative for MET expression.

For thyroid transcription factor-1 (TTF-1) staining, antigen retrieval was performed at boiling point in 1 mM ethylenediaminetetraacetic acid (pH 8.0). The slides were incubated with the primary monoclonal antibodies against TTF-1, clones 8G7G3/1 (dilution 1:75; Dako, Glostrup, Denmark) for 32 minutes at 37°C. All slides were counterstained with hematoxylin. Positive nuclear staining was defined as moderate to strong staining in the nuclei of any of the tumor cells

Statistical Analysis

Statistical analysis was performed using SPSS 19.0 (IBM Corp., Armonk, NY). Chi-square and Fisher's exact test were used to analyze associations of mutational, protein expression and gene copy number status with clinical characteristics. The Kaplan–Meier method was used to estimate the survival rates for different groups. The equivalences of the survival curves were tested by log-rank statistics. The Cox proportional hazards model was employed for univariate and multivariate survival analyses. Those variables being statistically significant found in the univariate survival analysis were further evaluated in the multivariate survival analysis.

A two-tailed p value of less than 0.05 was considered to be statistically significant.

RESULTS

Patient Characteristics

Frozen tumor tissues were available from a total of 166 patients who underwent surgery for lung cancer in the studied period. Among them 12 patients were excluded because of nonadenocarcinoma/adenosquamous carcinoma (n = 8), incomplete clinicopathological data (n = 3), and non-lung primary tumor (n = 1). The remaining 154 patients included 149 with lung adenocarcinoma and 5 with adenosquamous carcinoma. Eighty-six (55.8%) were male, and 68 (44.2%) were female. The mean age was 64.3 years (range 28–90 years). Among 149 patients with adenocarcinoma, most of the female subjects (80.6%) were never-smoker, whereas majority of the male subjects (63.4%) were ever-smokers ($p < 0.001$). According to the 7th edition of American Joint Committee on Cancer TNM staging, 69 patients (46.3%) were classified as stage I, 27 patients (18.1%) as stage II, 36 patients (24.2%) as stage III, and 17 patients (11.4%) as stage IV. For those patients underwent curative surgery (n = 132), resections were complete with margin clearance of more than 1 cm. Clinical characteristics of 149 patients with adenocarcinoma are displayed in Table 1. Details of the five patients with adenosquamous carcinoma were listed in Supplementary Table 2 (Supplemental Digital Content 1, http://links.lww.com/JTO/A853).

Mutation Spectrum in Lung Adenocarcinoma

Among 149 lung adenocarcinoma, 102 (68.5%) carried one driver mutation and two (1.3%) carried double mutations. A total of 106 driver mutations from 104 lung adenocarcinomas were identified. The remaining 45 (30.2%) tumors were found to be wild type from the genes we screened. The distribution of driver mutations is shown in Figure 1.

EGFR and KRAS were found to be frequently mutated in lung adenocarcinoma, and their mutations were mutually exclusive with each other. EGFR mutation was detected in 43% (64 of 149) of the adenocarcinomas. It was the most frequently mutated gene in this cohort. Exon 19 deletions (34 of 64, 53.1%) and exon 21 L858R point mutation (25 of 64, 39.1%) accounted for 92.2% of all detected EGFR mutations. Other EGFR mutations included E709_T710delinsD (n = 1) and E709A (n = 1) on exon 18, L747P (n = 1) on exon 19, H773Q (n = 1) on exon 20, and L861Q (n = 1) on exon 21. No EGFR exon 20 insertion was detected in this study. KRAS mutation was detected in 11.4% (17 of 149) of the adenocarcinomas. G12 residue was found to be affected in 16 cases, and Q61 residue was found to be affected in one case.

ALK translocation was also usually detected (9 of 149, 6%), as were MET exon 14 splice site mutations (6 of 149, 4%), ROS1 translocation (3 of 149, 2%), MET amplification (2 of 149, 1.3%), and BRAF V600E mutation (2 of 149, 1.3%). Other driver mutations in HER2 (1 of 149, 0.7%), NRAS (1 of 149, 0.7%), and PIK3CA (1 of 149, 0.7%) were observed. No mutation was detected in RIT1 and MAP2K1. Co-mutation was found in two tumors, one with NRAS (Q61H) and ALK translocation and the other with BRAF (V600E) and PIK3CA (E542K).

MET exon 14 splice site mutation was detected in six tumors. Two involved the deletion of the 5′ conserved polypyrimidine tract and the other four affected the 3′ splice-donor sites in intron 14. All these mutations were predicted to result in exon 14 skipping in MET mRNA (Fig. 2*A*). MET DNA amplification (MET/CEP7 ≥ 2) was found in two tumors (2 of 149, 1.3%, Fig. 2*B*). MET exon 14 splice site mutation and amplification were considered as oncogenic drivers that occurred mutually exclusively with other driver events of RTK/RAS/PI3K axis. Another five tumors were considered to have MET DNA copy number gain, which was defined by greater than or equal to five copies of MET DNA per cell. All five tumors with MET DNA copy number gain harbored co-mutations: two with coexisting MET exon 14 splice site mutations, one with KRAS mutation, and two with EGFR mutations.

MET IHC was successfully performed in 132 cases. Moderate to strong MET expression was found in 86 (65.1%)

TABLE 1. Clinicopathological Characteristics in Lung Adenocarcinoma

Parameter		Female (%)	Male (%)	Total
Age (yr)	Mean (SD)	63.4 (11.3)	64.8 (12.1)	—
	Range	34–82	28–90	—
Smoking status	NS	54 (69.2)	24 (30.8)	78
	ES	4 (7.1)	52 (92.9)	56
	NA	9 (60.0)	6 (40.0)	15
Pathological stage	I	29 (42.0)	40 (68.0)	69
	II	11 (40.7)	16 (59.3)	27
	III	21 (58.3)	15 (41.7)	36
	IV	6 (35.3)	11 (64.1)	17
Tumor size (cm)	Mean (SD)	3.1 (1.4)	3.75 (1.9)	—
	Range	1.0–8.0	1.5–10.0	-
Total		67 (45.0%)	82 (55.0%)	149

ES, ever smoker; NA, not available; NS, never smoker; SD, standard derivation.

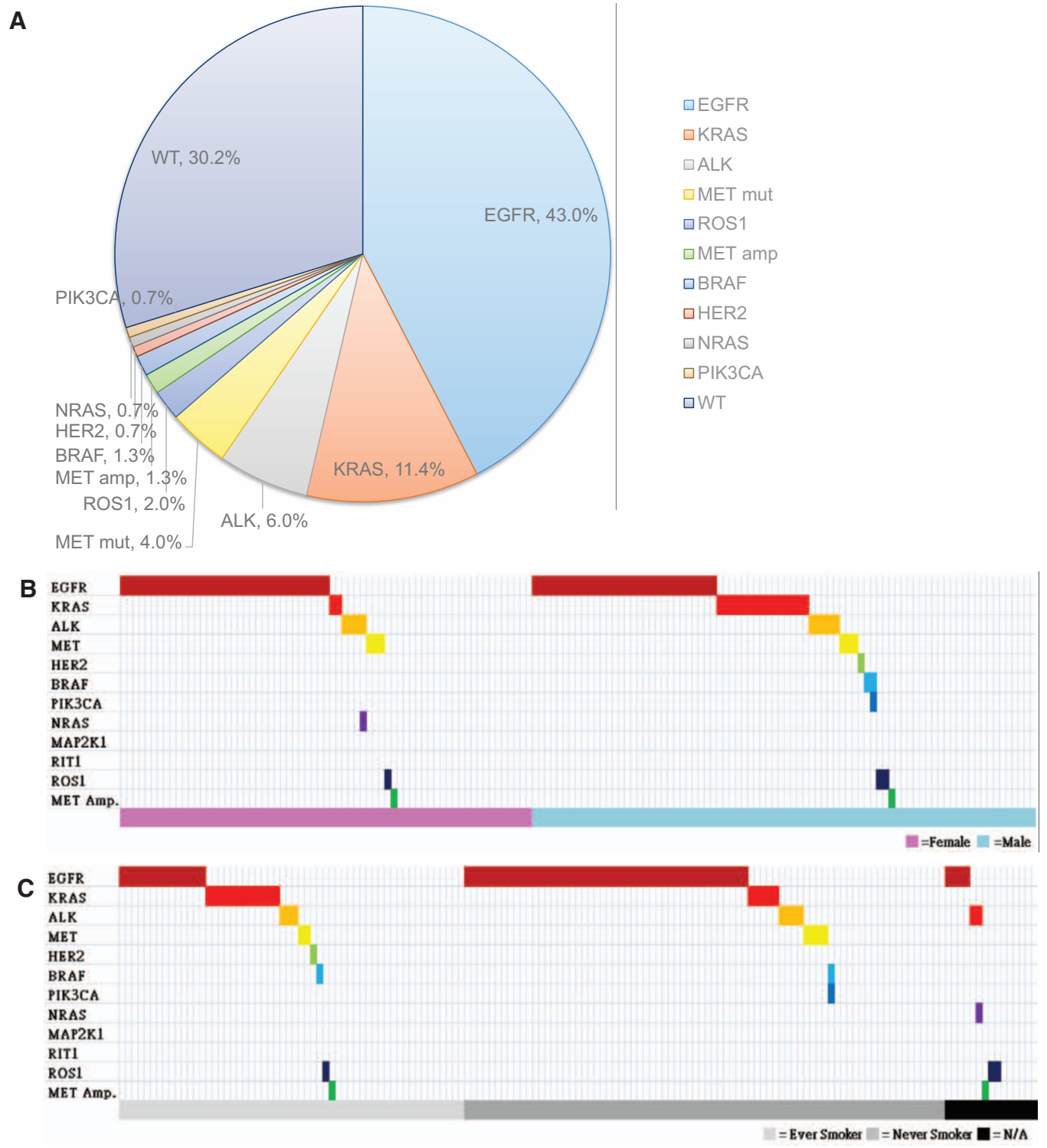

FIGURE 1. *A,* Frequencies of known oncogenic drivers on RTK/RAS/PI3K axis in lung adenocarcinoma (n = 149). Co-mutation plot for oncogenic driver stratified according to (*B*) gender and (*C*) smoking status.

tumors, whereas 46 (34.9%) cases were scored negative (Fig. 2*C*). All tumors with MET DNA alterations, including MET exon 14 splice site mutations, MET DNA amplification, and MET copy number gain were positive for MET protein expression. In 46 tumors that were negative for MET protein expression, no DNA alteration of MET gene was detected. Therefore, MET IHC had a 100% sensitivity (95% confidence interval [CI]: 71.3–100%) and negative predictive value

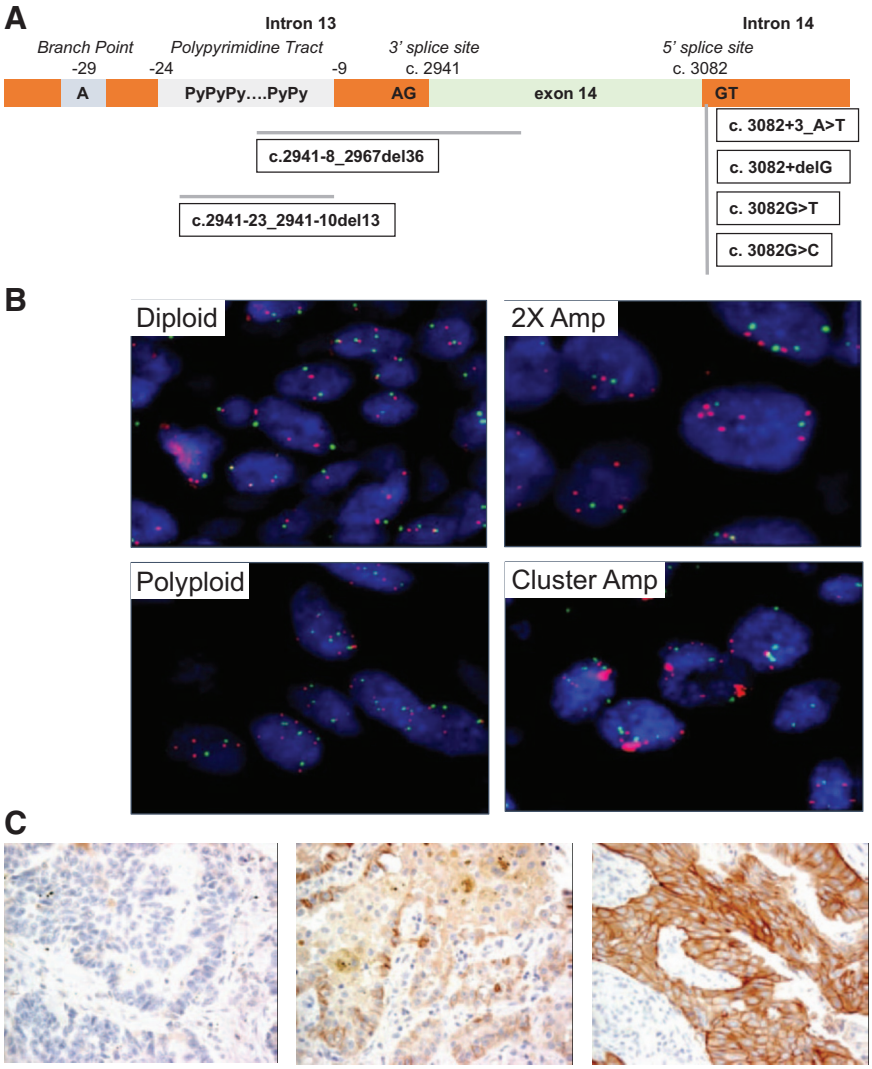

FIGURE 2. MET alterations in lung adenocarcinoma. *A,* Schematic illustration of the location of MET exon 14 splice site mutations found in this study. *B,* Representative images of MET FISH analysis. *C,* Representative images showing negative, moderate, and strong MET protein expression by immunohistochemistry.

(95% CI: 92.2–100%), though the specificity was 38% (95% CI: 29.4–47.3%) only, and the positive predictive value was 12.8% (95% CI: 6.6–21.8%).

Mutation Status in Lung Adenosquamous Carcinoma

Five cases of adenosquamous carcinoma were included in this study. Driver mutation was detected in two tumors, including one with EGFR exon 19 deletion and one with ALK translocation. Both patients were never smokers. No mutation was identified in the other three cases (Table 3, Supplemental Digital Content 1, http://links.lww.com/JTO/A853).

Correlation Between Driver Mutations and Clinicopathological Features

EGFR mutation rate was significantly higher in never-smokers than ever-smokers (59% versus 25%, $p < 0.001$), and in patients without lymph node metastasis ($p = 0.034$). There was a trend toward higher EGFR mutation rate in female than male (50.7% versus 36.6%, $p = 0.082$), although it was not statistically significant. No association was found between EGFR mutation status with patient's age, tumor size, and the pathological stages. On the contrary, KRAS mutations rate was significantly higher in ever-smokers than in never-smokers ($p = 0.016$) and in male patients ($p = 0.003$). ALK translocation was associated with younger age (52.7 versus 64.9 years, $p = 0.01$) and smaller tumor size ($p = 0.015$). Patients with MET exon 14 splice site mutations were significantly older (78.7 versus 63.6 years, $p = 0.001$), and the mutation status was correlated with MET DNA copy number gain (copy number ≥ 5, $p = 0.016$). Correlations of genotype with clinicopathological characteristics are listed in Table 2.

TTF-1 is a lineage specific maker that is uniformly expressed in the terminal respiratory unit (TRU) composed of peripheral airway cells and small-sized bronchioles. Given its role in lineage specification of lung epithelium, TTF-1 has been used as biomarker for primary lung adenocarcinoma. TTF-1 IHC was successfully performed in 138 cases. The results showed that 130 tumors (94.2%) were TTF-1 positive. The presence of TTF-1 expression positively correlated with EGFR mutation ($p = 0.021$) and negatively correlated with KRAS

TABLE 2. Correlation of EGFR, KRAS, and MET Mutations and ALK Translocation with Clinicopathological Features in Lung Adenocarcinoma

		EGFR Mutation			KRAS Mutation			ALK Translocation			MET Mutation		
Parameter		**Wild Type (%)**	**Mutant (%)**	***P* Value**	**Wild Type (%)**	**Mutant (%)**	***P* Value**	**Wild Type (%)**	**Fusion (%)**	***P* Value**	**Wild Type (%)**	**Mutant (%)**	***P* Value**
Gender	Male	52 (63.4)	30 (36.6)	0.082	67 (81.7)	15 (18.3)	0.003[b]	77 (93.9)	5 (6.1)	1.000	79 (96.3)	3 (3.7)	1.000
	Female	33 (49.3)	34 (50.7)		65 (97.0)	2 (3.0)		63 (94.0)	4 (6.0)		64 (95.5)	3 (4.5)	
Age (yr)	Mean (SD)	64.2 (12.4)	64.1 (10.9)	0.953	63.6 (12.1)	68.7 (7.4)	0.107	64.9 (11.3)	52.7 (14.2)	0.010[a]	63.6 (11.6)	78.7 (6.2)	0.001[b]
Smoking Status	NS	32 (41.0)	46 (59.0)	<0.001[b]	73 (93.6)	5 (6.4)	0.010[b]	74 (94.9)	4 (5.1)	1.000	74 (94.9)	4 (5.1)	1.000
	ES	42 (75.0)	14 (25.0)		44 (78.6)	12 (21.4)		53 (94.6)	3 (5.4)		54 (96.4)	2 (3.6)	
Pathological stage	IA–IIIA	71 (55.9)	56 (44.1)	0.499	112 (88.2)	15 (11.8)	1.000	120 (94.5)	7 (5.5)	0.622	121 (95.3)	6 (4.7)	0.592
	IIIB–IV	14 (63.6)	8 (36.4)		20 (90.9)	2 (9.1)		20 (90.9)	2 (9.1)		22 (100.0)	0 (0.0)	
Nodal metastasis	Positive	39 (67.2)	19 (32.8)	0.034[a]	53 (91.4)	5 (8.6)	0.449	52 (89.7)	6 (10.3)	0.092	84 (96.6)	3 (3.4)	0.683
	Negative	43 (49.4)	44 (50.6)		76 (87.4)	11 (12.6)		84 (96.6)	3 (3.4)		55 (94.8)	3 (5.2)	
Tumor size (cm)	Mean (SD)	3.5 (1.9)	3.4 (1.6)	0.895	3.4 (1.7)	4.1 (1.9)	0.054	3.5 (1.8)	2.3 (0.6)	0.015[a]	3.5 (1.8)	3.2 (0.6)	0.901
MET IHC	Negative	25 (32.1)	21 (38.9)	0.460	45 (47.1)	3 (18.8)	0.149	45 (36.3)	1 (12.5)	0.260	46 (36.5)	0 (0.0)	0.091
	Positive	53 (67.9)	33 (61.1)		73 (62.9)	13 (81.3)		79 (63.7)	7 (87.5)		80 (63.5)	6 (100.0)	
TTF-1 IHC	Negative	8 (10.0)	72 (90.0)	0.021[a]	4 (3.3)	4 (25.0)	0.007[b]	7 (5.4)	1 (11.1)	0.425	8 (6.1)	0 (0.0)	1.000
	Positive	0 (0.0)	58 (100.0)		118 (96.7)	12 (75.0)		122 (94.6)	8 (88.9)		124 (93.9)	6 (100.0)	

[a] $P < 0.05$.
[b] $P < 0.01$.
ES, Ever smoker; NA, not available; NS, Never smoker; SD, standard derivation; TTF-1, thyroid transcription factor-1; IHC, immunohistochemistry.

mutation ($p = 0.007$). Moreover, all TTF-1 negative tumors were negative for EGFR and MET mutations. Thus, the negative predictive values of TTF-1 for EGFR and MET mutations are 100% (95% CI: 62.9–100%). The finding suggested that TTF-1 might serve as a negative predictor for EGFR and MET mutations in lung adenocarcinomas (Table 3).

Survival Analysis

The follow-up data were available in 138 patients. The median follow-up time was 34.5 months. At the time of evaluation, 79 patients (57.2%) were still alive, whereas 91 patients (42.7%) had succumbed and 51 of them died of lung cancer. Advanced pathological stage, nodal metastasis, EFGR mutation, and MET mutation were prognostic factors of overall survival. Multivariate analysis revealed that advanced pathological stage, nodal metastasis, EFGR mutation, and MET mutation were independent prognostic parameters. Mutated EGFR was associated with better overall survival (hazard ratio, 0.49; 95% CI: 0.27–0.91; $p = 0.024$), whereas mutated MET was related to poorer overall survival (hazard ratio, 2.98; 95% CI: 1.03–8.67; $p = 0.045$).

Excluding 17 patients underwent palliative surgery, 71 patients (58.1%) were free of recurrence, and 51 (41.9%) had tumor relapse. The median duration of tumor relapse was 29.9 months. Nodal metastasis was the only prognostic factor predicting tumor relapse. None of molecular alteration was associated with tumor recurrence (Fig. 3 and Table 3).

DISCUSSION

Driver mutations in the RTK axis have been proven to be useful predictive biomarkers for targeted therapies that facilitate personalized medicine. Lung cancers demonstrate high rates of somatic mutation, and the list of actionable genetic alterations is growing during the past decade. Our study comprehensively investigated the frequencies and clinicopathological correlations of known actionable driver events in RTK/RAS/PI3K pathway in a Chinese cohort of lung adenocarcinoma. The results indicated that two thirds of the lung adenocarcinomas (70%, 104 of 149) carried actionable genetic events. EGFR and KRAS are the two most frequently mutated genes that occur in 43.0% and 11.4% of the tumors, respectively. Compared with ever-smokers, never-smokers had a higher EGFR mutation rate (59% in never-smoker versus 25% in ever-smoker) and lower KRAS mutation rate (6.4% in never smoker versus 21.4% in ever smoker). Notably, male and female never-smokers had a similar EGFR mutation rate (62% and 57%, respectively), indicating gender is not a selection criteria for EGFR testing. In contrast, male patients had higher proportions of KRAS mutations compared with female irrespective of smoking status.

No significant difference was observed between patient smoking history and gender in ALK, BRAF, and MET mutation status probably because of the limited sample size in this study. In addition, overall higher proportion of never-smokers tended to carry driver mutation-defined tumors than smokers though the difference did not reach statistical significance (77% versus 63%, $p = 0.07$). Individual mutations in RTK/RAS/PI3K pathway show specific demographic tendency, and therefore, similar studies could yield differential mutation spectrums. Our cohort composed of never-smoker predominated

TABLE 3. Univariate and Multivariate Cox Regression Analysis for Overall and Relapse-free Survival in Patients with Lung Adenocarcinomas

	Univariate Analysis			Multivariate Analysis		
Parameter	**Hazard Ratio**	**95% CI**	***P* Value**	**Hazard Ratio**	**95% CI**	***P* Value**
Overall survival						
Age (yr)	1.00	0.98–1.03	0.883			
Male	1.51	0.86–2.65	0.153			
Ever smoking	1.67	0.95–2.95	0.077			
Advanced pathological stage	4.60	2.49–8.48	<0.001[b]	4.85	2.55–9.22	<0.001[b]
Nodal metastasis[a]	2.60	1.48–4.59	0.001[b]			
Tumor size (cm)	0.93	0.77–1.13	0.460			
EGFR mutation	0.48	0.26–0.87	0.015[c]	0.49	0.27–0.91	0.024[c]
KRAS mutation	1.70	0.77–3.78	0.193			
ALK translocation	1.46	0.62–3.42	0.389			
MET mutation	2.93	1.05–8.21	0.041[c]	2.98	1.03–8.67	0.045[c]
Relapse free survival						
Age (yr)	1.00	0.97–1.02	0.878			
Male	1.25	0.71–2.20	0.444			
Ever smoking	1.17	0.66–2.07	0.589			
Advanced pathological stage	1.06	0.25–4.46	0.932			
Nodal metastasis	2.46	1.40–4.33	0.002[b]	2.46	1.40–4.33	0.002[c]
Tumor size (cm)	0.88	0.73–1.07	0.201			
EGFR mutation	0.82	0.47–1.45	0.498			
KRAS mutation	1.95	0.87–4.38	0.105			
ALK translocation	1.47	0.62–3.48	0.387			
MET mutation	1.51	0.36–6.28	0.573			

[a]Nodal metastasis was excluded for multivariate analysis to avoid multicollinearity with advanced pathological stage.
[b]$P < 0.05$.
[c]$P < 0.01$.
CI, confidence interval.

East Asian (Never-smoker to Ever-smoker ratio = 1.39). When comparing with other East Asian cohorts, the mutation pattern was more or less similar if stratified by smoking status,[14,26] However, the EGFR and KRAS shown reversed mutation rate in Caucasian population compare with our results regardless of never-smoker to ever-smoker ratio,[22,30] indicating ethnicity play important role in this discrepancy.

Driver mutation arises early during most tumor development, and single hit is enough to activate RTK pathway. Hence, they are usually mutually exclusive with each other.[31,32] However, tumors with double mutations are sometimes reported. Two lung adenocarcinomas in this cohort harbor co-mutations. One carries ALK translocations and NRAS mutation and the other harbors BRAF and PIK3CA mutations. Previous studies found PIK3CA mutations have a high chance to overlap with other mutations. Patients with double mutations show inferior survival.[33,34] Furthermore, overlapping PIK3CA/EGFR mutations contribute to resistance of EGFR TKIs. ALK translocations are also reported to coexist with EGFR or KRAS mutation. Patients with concomitant EGFR and ALK aberrations are responsive toward EGFR TKIs. The differential responses to targeting agents in tumors with co-mutations underscore the importance of comprehensive molecular profiling for lung adenocarcinoma.[35–37]

Prognostic significances of individual driver mutations have been investigated in many studies and summarized in different meta-analyses.[38] We demonstrated that mutated MET and wild-type EGFR were two independent prognostic factors of overall survival, but they did not predict the tumor recurrence. As majority of patients had disease beyond stage I, they are eligible for adjuvant systemic therapy according to current guidelines. The nature of systemic therapy could greatly influence overall survival. Although the data of adjuvant therapy was incomplete in our cohort, it is likely that advancement in EGFR-targeted therapy may explain that patients with EGFR mutation have a better overall survival than patients with MET mutation but not relapse-free survival.

Our study highlights potential therapeutic significance of MET DNA alteration in lung adenocarcinoma. MET DNA alterations define a subset of patients with older age and poorer prognosis that potentially might be beneficial by anti-MET targeting agents. Despite the positive MET protein expression that can be seen in 65% of lung adenocarcinoma, only 10% of these cases harbor MET DNA alterations that drive the overexpression of MET protein. Upregulation of MET protein in 90% of the MET IHC-positive tumors might be secondary events that promote tumor progression but not the key driver of individual tumors. Although

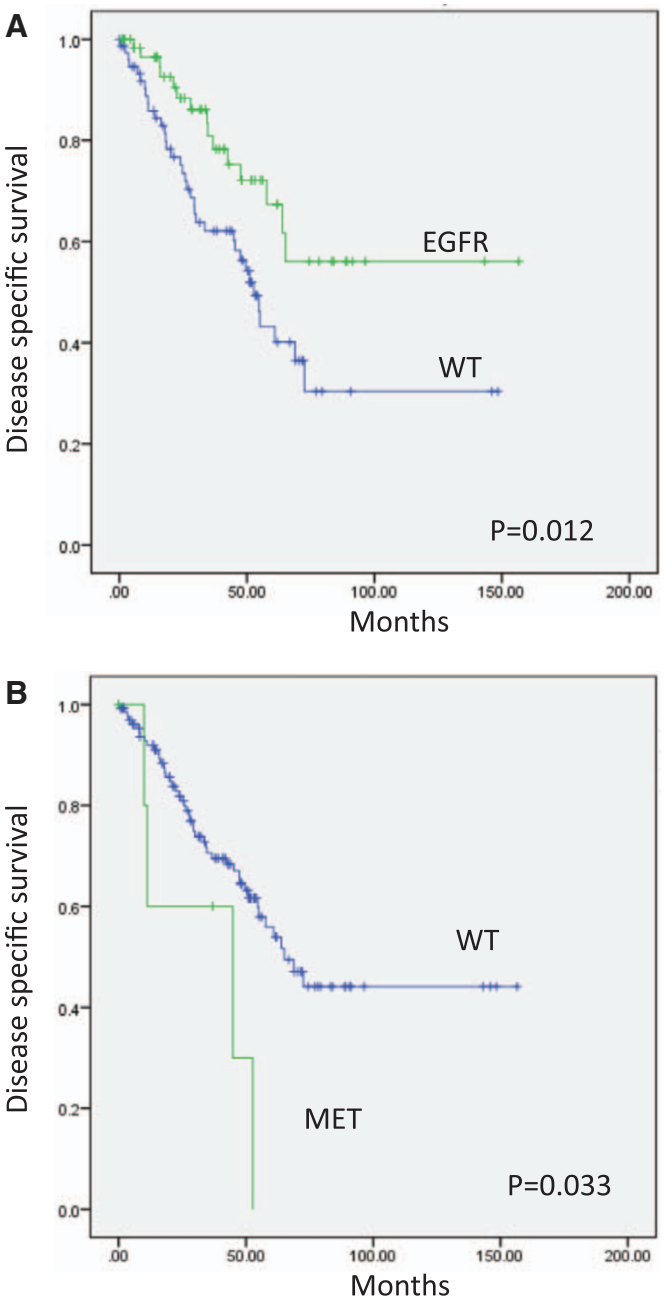

FIGURE 3. Kaplan–Meier survival curve for overall survival in lung adenocarcinoma according to (*A*) EGFR mutation and (*B*) MET mutation.

a recent study pooled over 5000 NSCLC cases for meta-analysis found MET protein overexpression predict poor prognosis in lung adenocarcinoma,[39] these tumors might not be response to anti-MET targeting agents. In fact, phase III clinical trials using MET protein expression as biomarker for patient selection failed to show improvement in overall and progressive-free survival (The MetLung and the MET Inhibitor Tivantinib (ARQ197) Plus Erlotinib vs Erlotinib Plus Placebo in NSCLC [MARQUEE] study). Our findings underscore the significance of proper selection of predictive biomarkers, which is essential for patient management in the new era of personalize medicine. The high negative predictive value of MET protein expression level for the presence of MET DNA alterations is intriguing. MET IHC is conveniently available in routine diagnostic laboratory that allows for a fast screening for patients with lung adenocarcinoma to join proper molecular test.

Lack of TTF-1 expression in lung adenocarcinoma has been associated with higher KRAS and lower EGFR mutation rate in previous studies.[40,41] It was proposed that lung adenocarcinoma could be classified into TRU subtype and non-TRU subtypes principally according to TTF-1 expression.[42] TRU cell lines are more sensitive to EGFR TKIs, whereas non-TRU cell lines are more sensitive toward cisplatin regardless of KRAS status. In this study, a high negative predictive value of TTF-1 for the presence of EGFR and MET mutation suggested a potential role for TTF-1 as a surrogate marker.

In conclusion, actionable driver events can be detected in two thirds of lung adenocarcinoma from Chinese ethnicity. MET DNA alterations define a subset of patients with aggressive diseases that might potentially benefit from anti-MET targeted therapy. High negative predictive values of TTF-1 and MET expression suggest potential roles as surrogate markers for EGFR and/or MET mutations.

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